COMPARISON OF GRAPHITIZED CARBON BLACK AND A NOVEL SORBENT IN DISPERSIVE-SPE CLEANUP OF SPINACH EXTRACT

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1. INTRODUCTION

Spinach is a highly pigmented matrix containing chlorophyll, carotenoid, xanthophyll and anthocyanin pigments. Among these, chlorophyll pigments have the greatest adverse effect on GC systems due to their non-volatile characteristics. When a sample containing chlorophyll is injected into a GC, chlorophyll will accumulate in the GC inlet and on the front end of the column, generating active sites and affecting GC performance. Therefore, it is essential to remove chlorophyll prior to analysis.

The two most commonly used sorbents in the dispersive solid-phase extraction (dSPE) cleanup step of the QuEChERS method include primary secondary amine (PSA) and endcapped C_{18}. PSA is effective for removal of acids and polyphenolic anthocyanin, while C_{18} effectively removes lipids, carotenoid and xanthophyll. An alternative sorbent widely used for the effective cleanup of highly pigmented matrices, including those containing chlorophyll, is graphitized carbon black (GCB). However, while GCB is effective for purifying samples, a major disadvantage is its adsorption of target analytes that contain planar structures, resulting in low recovery of these compounds. Examples of planar pesticides include carbendazim, thiabendazole, pyrimethanil, and cyprodinil. To release planar analytes from the GCB sorbent, toluene can be added to the sample extract before dSPE cleanup. However, additional evaporation and reconstitution steps are needed to remove toluene prior to LC analysis, as toluene causes solvent immiscibility that leads to peak shape issues. Many laboratories have reduced their use of toluene due to its health risks.

To overcome the problems caused by use of GCB and to avoid the use of toluene, UCT has developed a novel polymeric sorbent, named ChloroFiltr®, for the removal of chlorophyll from highly pigmented samples without sacrificing the recovery of planar analytes. This poster details a study that compared the recovery of planar pesticides and extract cleanliness of fortified spinach samples that underwent dSPE cleanup with ChloroFiltr® and GCB.

Carbendazim
CAS 10605-21-7
pKa = 4.88

Thiabendazole
CAS 148-79-8
pKa = 4.64

Pyrimethanil
CAS 53112-28-0
pKa = 3.52

Pyrazophos
CAS 13457-18-6
pKa not reported

Cyprodinil
CAS 121552-61-2
pKa = 4.44

Diazinone
CAS 333-41-5
pKa = 2.6

Chlorpyrifos
CAS 29121-88-2
pKa = 4.55

Figure 1. Chemical structures of the seven pesticides included in this study

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2. EXPERIMENTAL

2.1. Materials

100 ppm carbendazim, thiabendazole, pyrimethanil, cyprodinil, and pyrazophos standards in methanol were purchased from Ultra Scientific (N. Kingstown, RI). 100 ppm chlorpyrifos and diazinone standards in methanol were purchased from Chem Service (West Chester, PA). A 5000 ppm solution of triphenyl phosphate (TPP) in methyl tert -butyl ether was purchased from Cerilliant (Round Rock, TX). HPLC grade acetonitrile (MeCN) and methanol (MeOH) were purchased from Spectrum (New Brunswick, NJ). The QuEChERS extraction and dSPE cleanup materials were supplied by UCT (Bristol, PA) and are listed in the table below.

Table 1. UCT materials for QuEChERS extraction and dSPE cleanup

50 mL tubes (UCT part#: RFV0050CT)	50 mL polypropylene centrifuge tubes
Extraction salts (UCT part#: ECQUUS2-MP)	Mylar pouch with 4000 mg $MgSO_4$ and 2000 mg NaCl
dSPE cleanup tubes with GCB (UCT part#: CUMPSC1875CB2CT)	2 mL centrifuge tube with 150 mg $MgSO_4$, 50 mg PSA, 50 mg C_{18}, and 7.5 mg GCB
dSPE cleanup tubes with ChloroFiltr®	2 mL centrifuge tube with 150 mg $MgSO_4$, 50 mg PSA, 50 mg C_{18}, and various amounts of ChloroFiltr®

2.2. Preparation of standard solutions

Pesticide Spiking Solution
A 5 ppm pesticide spiking solution was prepared by adding 500 µL of the seven 100 ppm pesticide standards into a 10-mL volumetric flask and diluting to volume with MeCN. A 0.5 ppm mixed pesticide standard solution was prepared by adding 1 mL of the 5 ppm pesticide spiking solution into a 10-mL volumetric flask and diluting to volume with MeCN.

Internal Standard triphenyl phosphate (TPP)
A 50 ppm TPP internal standard (IS) solution was prepared by mixing 50 µL of the 5000 ppm TPP solution with 5 mL of MeCN.

All standards were stored in amber glass vials with Teflon lined caps in freezer (-20ºC) until use.

2.3. Procedure

2.3.1. QuEChERS extraction

1. 500 g of fresh spinach purchased from a local grocery store was homogenized using a food processor.
2. 10 g of the homogenized spinach sample was weighed into 50 mL centrifuge tubes.
3. Spike all fortified and blank control samples with 100 µL of the 50 ppm TPP IS solution.
4. Add appropriate amounts of the 5 ppm pesticide spiking solution to the fortified samples.
5. Vortex for 30 sec and equilibrate for 15 min.
6. Add 10 mL of MeCN and vortex for 1 min.
7. Add salts packed in a Mylar pouch containing 4000 mg $MgSO_4$ and 2000 mg NaCl, shake vigorously for 1 min.
8. Centrifuge at 5000 rpm for 5 min. The supernatant is ready for cleanup.

2.3.2. dSPE cleanup

1. Transfer 1 mL of the supernatant into 2 mL dSPE tubes containing different amounts of sorbents (listed in Table 2) and shake for 30 sec.
2. Centrifuge at 10,000 rpm for 5 min.
3. Transfer 0.5 mL of the cleaned extracts into 2-mL autosampler vials.
4. The samples are ready for LC/MS/MS analysis.

Table 2 Sorbent components in dSPE tubes for spinach cleanup

dSPE tube	$MgSO_4$ (mg)	PSA (mg)	C18 (mg)	GCB (mg)	ChloroFiltr® (mg)
A	150	50	50	0	7.5
B	150	50	50	0	15
C	150	50	50	0	25
D	150	50	50	0	50
E	150	50	50	7.5*	0

*7.5 mg GCB is chosen for the cleanup of dark green extracts as stated in Method EN 15662.

2.4. Matrix-matched calibration curves

Matrix-matched calibration curves were generated from the blank spinach extracts that were prepared by QuEChERS extraction and dSPE cleanup with 150 mg $MgSO_4$, 50 mg PSA, 50 mg C18 and 50 mg ChloroFiltr®. Appropriate volumes of the 0.5 and 5 ppm pesticide standard solutions were spiked into the sample extracts to generate 6-point calibration curves with concentrations of 2, 10, 40, 100, 200, and 400 ng/mL.

2.5. Instrumental conditions

Table 3. HPLC conditions

HPLC system	Thermo Accela 1250 LC equipped with a PAL
LC Column	Sepax HP-C18, 2.1*100 mm, 3 µm
Guard column	Restek C18, 2.1*20 mm, 3 µm
Column temperature	Ambient
Autosampler	15 °C
Injection volume	10 µL
Mobile phase A	0.1% formic acid in Milli-Q H_2O
Mobile phase B	0.1% formic acid in MeOH
Flow rate	200 µL/min
Gradient program	
Time (min)	**Mobile phase A**	**Mobile phase B**
0	95	5
1	95	5
3	50	50
8	5	95
14	5	95
14.2	95	5
16	95	5

Table 4. MS Parameters

MS instrument	Thermo TSQ Vantage triple
Polarity	ESI +
Spray Voltage	3000 V
Vaporizer Temperature	350 °C
Ion Transfer Capillary Temperature	300 °C
Sheath Gas Pressure	40 arbitrary units
Auxiliary Gas Pressure	10 arbitrary units
Q1 and Q3 Peak Width (FWHM)	0.7 Da
Collision Gas and Pressure	Argon at 1.5 mTorr
Dwell time	100 ms

Table 5. SRM transitions

Compound	Precursor ion	Product ion 1	CE 1	Product ion 2	CE 2	S-Lens
Carbendazim	192.093	132.060	29	160.080	17	81
Thiabendazole	202.059	131.060	31	175.070	31	103
Pyrimethanil	200.116	107.060	23	183.140	22	66
Cyprodinil	226.122	77.030	40	93.050	33	88
TPP (IS)	327.093	77.020	37	152.070	33	98
Diazinone	305.135	153.060	15	169.08	14	89
Pyrazophos	374.103	194.060	20	222.130	26	104
Chlorpyrifos	349.989	96.890	32	197.940	17	69

3. RESULTS

3.1. Comparison of ChloroFiltr® vs. GCB on planar pesticide recovery

Spinach samples fortified with 50 ng/g pesticides were extracted and cleaned up with dSPE tubes containing 150 mg $MgSO_4$, 50 mg PSA, 50 mg C_{18}, and either 50 mg ChloroFiltr®, or 7.5 mg GCB. ChloroFiltr®, gave good recoveries for all 7 pesticides. The recoveries of carbendazim, cyprodinil, pyrimethanil and thiabendazole were adversely affected by GCB, with thiabendazole in particular obtaining much lower recovery with GCB (56% vs. 93% with ChloroFiltr®,). Diazinone, pyrazophos, and chlorpyrifos were unaffected by GCB due to the presence of non-planar side chains in their structures.

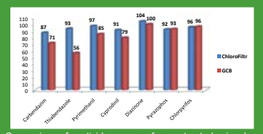

Figure 2. Comparison of pesticide recovery from extracted spinach samples purified by dSPE with ChloroFiltr®, and GCB

3.2. Visual assessment of extract cleanliness

After undergoing cleanup with a variety of different sorbent combinations **(Table 2)**, spinach extracts were visually assessed for sample cleanliness and chlorophyll removal. As can be seen in Figure 3, the spinach extract decreases in chlorophyll content as the quantity of ChloroFiltr®, is increased (A-D). Although GCB (E) is effective in removing chlorophyll, it contains slightly more chlorophyll than that cleaned with 50mg ChloroFiltr®, and results in low recovery of planar pesticides. Therefore, dSPE tube containing 150 mg $MgSO_4$, 50 mg PSA, 50 mg C_{18} and 50 mg ChloroFiltr® (UCT part #: CUMPSGCC182CT) was selected for spinach cleanup as it gave efficient chlorophyll removal and excellent pesticide recoveries.

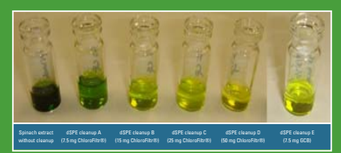

Figure 3. Visual comparison of extract cleanliness after dSPE cleanup with different amounts of ChloroFiltr®, and 7.5 mg GCB

3.3. Recovery study

The spinach sample that confirmed the absence of the target pesticides was used for recovery study at three spiking levels: 10, 50, and 100 ng/mL. The fortified spinach samples were extracted using a QuEChERS approach and cleaned up with dSPE containing 150 mg $MgSO_4$, 50 mg PSA, 50 mg C_{18} and 50 mg ChloroFiltr®. The recoveries ranged from 87.1 to 108.7% with relative standard deviations (RSD) less than 6.1%, indicating that this method is suitable for the analysis of planar pesticides in spinach samples.

Table 6. Accuracy and Precision Data

Compound	Fortified at 10 ng/g		Fortified at 50 ng/g		Fortified at 100 ng/g	
	Recovery%	RSD% (n=4)	Recovery%	RSD% (n=4)	Recovery%	RSD% (n=4)
Carbendazim	94.9	6.1	87.1	1.0	95.1	2.0
Thiabendazole	94.2	5.8	93.2	1.9	98.2	3.1
Pyrimethanil	100.0	4.1	97.3	1.2	94.8	2.6
Cyprodinil	98.6	1.0	91.2	0.5	87.2	1.7
Diazinone	108.7	3.6	104.5	2.3	100.2	1.0
Pyrazophos	99.7	2.9	92.0	0.9	87.7	2.1
Chlorpyrifos	100.9	5.5	95.6	2.5	94.9	1.8
Mean	**99.6**	**4.1**	**94.4**	**1.5**	**94.0**	**2.0**

3.4. Chromatogram

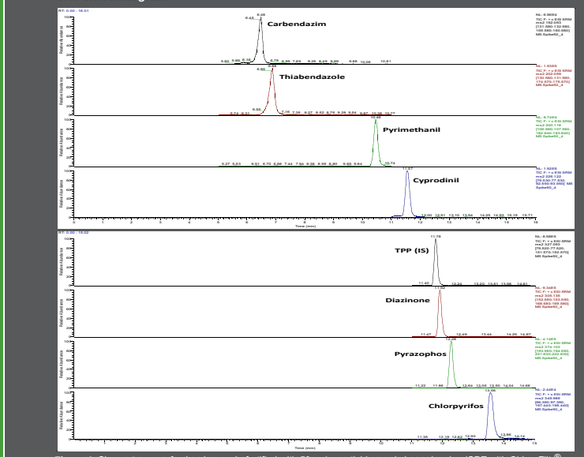

Figure 4. Chromatogram of spinach sample fortified with 50 ng/g pesticides and cleaned up by dSPE with ChloroFiltr®.

3.5. Matrix-matched calibration curves

The response of the seven matrix-matched calibration curves were found to be linear over the 2-400 ng/mL concentration range. The correlation coefficients (R^2) were better than 0.9982. The limit of quantification (LOQ) of the method was found to be 2 ng/g for all seven pesticides.

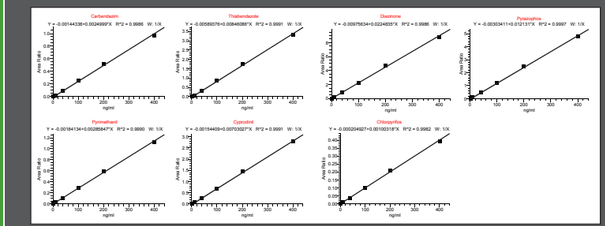

Figure 5. Calibration curves of the seven pesticides in this study

4. CONCLUSIONS

A simple, sensitive, and effective method was developed for the determination of planar pesticides in spinach samples. Spinach samples were extracted using the original QuEChERS approach and cleaned up by dSPE containing $MgSO_4$, PSA, endcapped C_{18} and ChloroFiltr®. ChloroFiltr® is a novel sorbent that effectively removes chlorophyll without sacrificing the recovery of planar pesticides, offering a successful substitute for GCB in cleanup of samples containing high chlorophyll levels.

QR Code